Chapter 5

Summary

5.1 English summary

The cell is the basic structural part of all living matter on Earth. The shape and size of a single cell vary tremendously, however, a common feature shared by all cells is that the interior is separated from the environment by a membrane. The interior, or cytoplasm, contains all the machinery for cell growth, metabolic functions and the genetic information contained in DNA. To support life, a cell needs to take up nutrients from its environment and secrete products. However, most nutrients need specialized channels in the membrane to pass this barrier. These specialized channels are protein (complexes) embedded in the membrane, which are inserted into this membrane by a highly conserved protein complex termed Sec61 for Eukaryotes and SecYEG for Bacteria. The heterotrimeric SecYEG complex of the cytoplasmic membrane of bacteria, consist of the SecY, -E and –G proteins and facilitates the passages of proteins across and into this membrane. During the past decades structural and biochemical studies have revealed a complex pathway centered around the SecYEG translocon. This pathway consists of two dedicated sub routes for membrane and secretory proteins, although there are exceptions to this division. These subroutes contain dedicated proteins that work in concert to facilitate efficient protein insertion and translocation in a super complex called the holotranslocon. Our knowledge of the protein insertion and translocation mechanisms today is primarily based on observations via biochemical assays. However, the idiom "seeing is believing" has a strong impact on us humans and since the emergence of powerful microscopes, also on the field of science as well. Being able to visualize single molecules and describing their characteristics and dynamics, often provides tremendous new insights. In this thesis, I used single-molecule fluorescence microscopy to investigate different aspects of the bacterial secretion system. This technique is perfectly suited to answer long standing questions, which were only addressed previously using biochemical assays and which have led to various controversies; what is the exact location of the components of the bacterial secretion system in a living cell? What is their functional state under native condition? How do the components dynamically localize in living cells? **Chapter 1** presents the reader with a detailed overview of the protein secretion pathway. It introduces key open questions and explains how structural, biochemical and single-molecule techniques are used to address them. Furthermore, it provides a review of recent single-molecule studies on the bacterial secretion pathway and provides a few examples of the single-molecule experiments used in this thesis.

Chapter 2 forms the experimental and technical basis of this thesis with the focus on the ATPase motor protein of the *Escherichia coli* secretion pathway, SecA. In this chapter, principles underlying the analysis of the fluorescent data were formed and optimized. One example is the use of a refined way of analyzing the movement of particles, making it possible to distinguish multiple diffusive populations. The analysis

routine developed in this chapter, revealed the location of fluorescently labeled SecA below the diffraction limit being almost exclusively located at the cytoplasmic membrane and demonstrated the homodimeric nature of SecA in the cell. Furthermore, multiple populations of diffusive molecules were found, the most parsimonious hypothesis is, that these populations arise from interactions with other proteins like the holotranslocon.

Chapter 3 is dedicated to finding irrefutable evidence for the existence of the elusive holotranslocon. There is only circumstantial evidence for the presence of such super complex *in vivo*, however, the single-molecule imaging method and analysis developed in chapter 2 allowed us to visualize components constituting the holotranslocon simultaneously. The *in vivo* data presented in this chapter suggests that the holotranslocon is a dissociable entity and evidence is provided for various subcomplexes that diffuse with different rates.

Chapter 4 isolates our focus on the binding effects of substrates to the prokaryotic SecYEG translocon. A single-molecule *in vitro* approach utilizing a model membrane system was taken to visualize the diffusive behavior of fluorescently labeled SecYEG. A method to create supported lipid bilayers with almost native like lipid composition was developed to image single SecYEG molecules diffusing through it or order to provide a benchmark for the membrane diffusion of defined complexes containing SecYEG**.**

5.2 Peroration

To this day, the Sec-translocon is still a topic of intensive study. Biochemical and structure elucidation studies performed over the past decades, provide tremendous insights into the mechanistically workings of proteins and protein complexes, however, transient, fast and dynamical information is often lost in these approached due to ensemble averaging. Super-resolution microscopy techniques overcome this shortcoming by focusing on and measuring at the single-molecule level.

In this thesis I employed a PALM-type super-resolution microscopy technique to visualize different components of the prokaryotic Sec system on the single-molecule level in living cells and extracting information not observed before. Obtaining such detailed information on these essential biological processes was only made possible due to the rapid increase in our collective knowledge of single-molecule techniques over the past decades. The ongoing development of new, as well as the refinement of existing single-molecule techniques and methods will pave the way to an even more detailed and complete understanding of the molecular mechanisms and processes sustaining life. Therefore, single-molecule approaches will play an increasingly larger role to study complex biological processes both *in vitro* and *in vivo*, leading to groundbreaking new insights and the prokaryotic Sec pathway will be no exception. The developed methods in this thesis will be used to further study the Sec components and visualize even more details about the formation of the holotranslocon.